

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT Application of  
Saville, et. al.

Group Art Unit: 1651

U.S. Serial No. 10/797,019

Examiner: Gough, Tiffany Maureen

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Att. Docket No.: 95773-1439

For: ENHANCEMENT OF ENZYME ACTIVITY BY SELECTIVE PURIFICATION

\* \* \* \* \*

24 April 2007

**RULE 132 DECLARATION**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Bradley A. Saville, declare and state as follows:

1. I am currently employed by \_The University of Toronto\_\_\_\_\_. My present position and title are \_Professor of Chemical Engineering and Applied Chemistry\_\_\_\_\_, \_\_\_\_\_.

2. I have been continuously employed since 1989\_\_ with the University of Toronto\_\_\_\_\_. During that time I have held the following positions: \_\_\_\_\_: \_Assistant Professor, Associate Professor, Full Professor; Coordinator of Occupational Health and Safety\_\_\_\_\_.

(Here provide a brief employment history and positions or titles held at each company)

**ACADEMIC AND PROFESSIONAL EXPERIENCE:**

1989 - 1994, Assistant Professor, University of Toronto

1994 - 2000, Associate Professor, University of Toronto

2000 - Present, Full Professor, University of Toronto

1991 - Present, Consultant and Director, Chemical Engineering Research Consultants Ltd.

2001 - Present, Consultant and President, 1484667 Ontario Ltd.

2003 - Present, Scientific Advisor and Director, Immortazyme Company

2005 - Present, Technical Advisor, BBI International and BBI Biofuels Canada

3. I am the author or co-author of many articles related to the field of biotechnology and bioprocess engineering \_\_\_\_\_. A list of these articles is shown in Attachment 1.

4. I am the inventor or co-inventor of many inventions related to the field of biotechnology and bioprocess engineering \_\_\_\_\_. A list of these patents or patent applications is shown in Attachment 2.

5. I have read and understood the present patent application. I have also read and understood the Office Actions and the cited prior art in the present patent application.

6. I performed or supervised the following experiments.

7. In Figure 1, I compared the activity of native Ligozyme SC DS®, a commercial amylase produced by Novozymes, with that of Ligozyme processed through activated carbon (Processed Liq. SCDS) and that of amylase produced using

the novel process disclosed in the present application (IMA from Ligozyme SCDS). The IMA was prepared by first diluting Ligozyme® 10-fold with an aqueous buffer solution, and then passing the diluted enzyme through a column containing activated carbon.

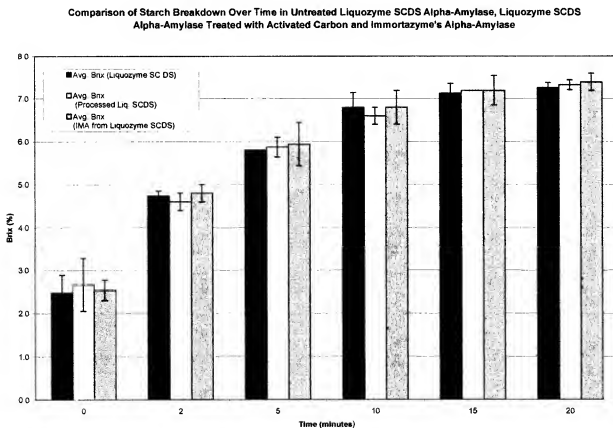


Fig. 1: Impact of Activated Carbon and Activated Carbon + Dilution on Activity of Ligozyme SC DS®

8. In Figure 2, I compared the activity of native Ultrathin L100®, a commercial amylase produced by Valley Research, with that of Ultrathin processed through activated carbon (Processed Ultrathin) and that of amylase produced using the novel process disclosed in the present application (IMA from Ultrathin). The IMA was prepared by first diluting Ligozyme® 10-fold with an aqueous buffer solution, and then passing the diluted enzyme through a column containing activated carbon.

Comparison of Starch Breakdown Over Time in Untreated UltraThin L100 Alpha-Amylase, Ultrathin L100 Alpha-Amylase Treated with Activated Carbon and Immortazyme's Alpha-Amylase

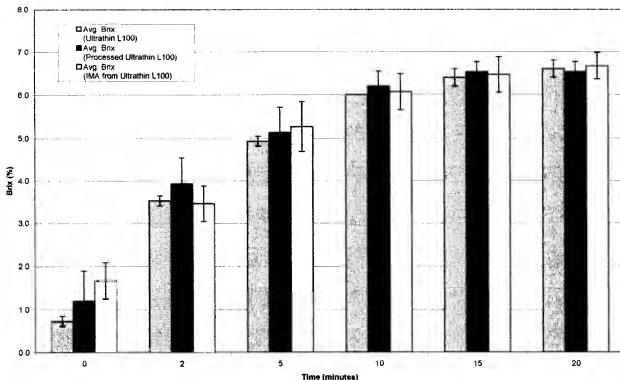


Fig. 2: Impact of Activated Carbon and Activated Carbon + Dilution on Activity of Ultrathin L100®

9. Comparing the profiles for Processed Ultrathin and Processed Liquozyme to those for their untreated counterparts demonstrates that simply contacting these amylases with activated carbon has no impact on their activity (Figures 1 and 2). These results, therefore, provide further evidence that processing with activated carbon alone does not automatically enhance enzyme activity, contrary to the Examiner's assertions.

10. Unexpectedly, the activity of the IMA samples is equivalent to that obtained with the untreated, undiluted enzyme solution, even though the IMA samples contain only 1/10<sup>th</sup> of the total protein present in the original enzyme solution. Expressing the activity on a per unit mass protein basis, it is apparent that there has been a surprising 10-fold increase in activity after the enzyme has been processed according to the

present novel process. If there was no 10-fold increase in activity, the left most bar would only be 1/10 the height of the center and right most bar for each three bar set in Figs 1 and 2. This improvement is clearly not realized by simply processing with activated carbon, but it can be obtained when a sufficiently dilute enzyme sample is processed through activated carbon, using the present novel process.

11. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: \_\_\_\_\_



Bradley A. Saville

Date: April 26, 2007

**SUMMARY OF RESEARCH CONTRIBUTIONS (TO OCTOBER 2006)**

	Refereed Journals	Conference Proceedings and abstracts	Books; Chapters	Technical Reports	Patents
Last 5 Yrs	11	9	2	14	13
Total	32	14	2	16	14

**Attachment #1: List of publications**

1. Saville, B.A., Gray, M.R., and Tam, Y.K., "The Metabolism of Lidocaine in the Liver: Steady State and Dynamic Modelling", *CJChE*, 64, 617-624, 1986.
2. Saville, B.A., Gray, M.R., and Tam, Y.K., "Mechanisms of Lidocaine Kinetics in the Isolated Perfused Rat Liver. II. Kinetics of Steady State Elimination", *Drug Metabolism and Disposition*, 15(1), 17-21, 1987.
3. Gray, M.R., Saville, B.A., and Tam, Y.K., "Mechanisms of Lidocaine Kinetics in the Isolated Perfused Rat Liver. III. Evaluation of Liver Models for Time-Dependent Behaviour", *Drug Metabolism and Disposition*, 15(1), 22-26, 1987.
4. Saville, B.A., Gray, M.R., and Tam, Y.K., "Evidence for Lidocaine-Induced Enzyme Deactivation", *Journal of Pharmaceutical Sciences*, 78(12), 1003-1008 (1989).
5. Saville, B.A., Gray, M.R., and Tam, Y.K., "Models of Hepatic Drug Elimination", *Drug Metabolism Reviews*, 24(1), 49-88 (1992).
6. Saville, B.A., Gray, M.R., and Tam, Y.K., "Experimental Studies of Transient Mass Transfer and Reaction in the Liver: Interpretation Using a Heterogeneous Compartment Model", *J. Pharm. Sci.*, 81(3), 265-272 (1992).
7. Saville, B.A., and Persi, S., "The Effect of Oxygen upon the Kinetics of Enzyme Inactivation: In Vitro Investigations Using Glutamine Synthetase", *Can. J. Chem. Eng.*, 70, 1143-1148 (1992).
8. Friedrich, S.F., Cheng, Y-L. and Saville, B.A., "Theoretical Corneal Permeation Model for Drugs in Ionic and Non-Ionic Form", *Inv. Ophth. Vis. Sci.*, 34(4), 854 (1993).
9. Oh, C., Apel, A., Chiu, R., Saville, B.A. and Cheng, Y-L., "An Ocular Emphasized Multi-Compartment Model for Cyclosporine: An Application in the Therapy of Corneal Transplantation", *Inv. Ophth. Vis. Sci.*, 34(4), 1490 (1993).
10. Apel, A., Oh, C., Chiu, R., Cheng, Y-L., Saville, B.A. and Rootman, D., "Cyclosporine Delivery Using a Subconjunctival Biodegradable Polymer Device", *Inv. Ophth. Vis. Sci.*, 34(4), 1489 (1993).
11. Venugopal, R. and Saville, B.A., "The Effect of Oxygen Upon the Kinetics of Glucose Oxidase Inactivation", *Can. J. Chem. Eng.*, 71, 917-924 (1993).
12. Oh, C., Apel, A., Saville, B.A., Cheng, Y-L. and Rootman, D.S., "Local Efficacy of Cyclosporine in Corneal Transplant Therapy", *Current Eye Research*, 13, 337-343 (1994).
13. Chiu, R. and Saville, B.A., "Effect of Oxygen upon the Kinetics of Tyrosinase Inactivation", in *Food and Bioproducts Processing*, *Trans. IChemE*, 72(C), 41-46, (1994).

14. Oh, C., Saville, B.A., Cheng, Y-L. and Rootman, D.S., "A Compartmental Model for the Ocular Pharmacokinetics of Cyclosporine in Rabbits", *Pharm. Res.*, 12, 433-437 (1995).
15. Apel, A., Oh, C., Chiu, R., Saville, B.A., Cheng, Y-L. and Rootman, D.S., "A Subconjunctival Degradable Implant for Cyclosporine Delivery in Corneal Transplant Therapy", *Current Eye Research*, 14, 659-667 (1995).
16. Friedrich, S., Saville, B.A., Cheng, Y-L. and Rootman, D.S., "Pharmacokinetic Differences Between Ocular Inserts and Eyedrops", *J. Ocular Pharmacology and Therapeutics*, 12, 5-18 (1996).
17. Jimenez Hamann, M.C. and Saville, B.A., "Enhancement of Tyrosinase Stability by Immobilization on Nylon 66", *Trans. IChemE.*, 74(c), 47-52 (1996).
18. Pialis, P., Jimenez Hamann, M.C. and Saville, B.A., "L-DOPA Production for Tyrosinase Immobilized on Nylon 66", *Biotechnol. Bioeng.*, 51, 141-147 (1996).
19. Friedrich, S., Saville, B.A. and Cheng, Y-L., "Finite Element Modelling of Drug Distribution in the Vitreous Humour of the Rabbit Eye", *Annals of Biomedical Engineering*, 25, 303-314 (1997).
20. Friedrich, S., Saville, B.A. and Cheng, Y-L., "Drug Distribution in the Vitreous Humour: Effects of Aphakia and Changes in Retinal Permeability and Diffusivity", *J. Ocul. Pharmacol. Ther.*, 13(5), 445-450 (1997).
21. Friedrich, S., Saville, B.A. and Cheng, Y-L., "Drug Distribution the Vitreous Humour: Effects of Injection Position and Injection Volume", *Curr. Eye Res.*, 16, 663-669 (1997).
22. Pialis, P. and Saville, B.A., "Production of L-Dopa from Tyrosinase Immobilized on Nylon 6,6: Enzyme Stability and Scale-Up", *Enzyme and Microbial Technology*, 22, 261-268 (1998).
23. Warrington, J.C. and Saville, B.A., "The Effect of a Perfluorochemical Emulsion on Enzyme Reaction Stability", *CJChE*, 77, 877 - 882 (1999).
24. Warrington, J.C., and Saville, B.A., "Tyrosinase Inactivation in Organic Solvents", *Biotechnol. Bioeng.*, 65, 325-333, (1999).
25. Ng, C, Cheng, Y-L., and Saville, B.A., "Thermal Responsive Polymer Membrane for the Local Delivery of Drugs", *J. Sex. Reprod. Med.*, 1(1), 21-27 (2001).
26. Seetharam, G., and Saville, B.A., "L-DOPA Production from Tyrosinase Immobilized on Zeolite", *Enz. Microb. Technol.*, 31, 747-753 (2002).
27. Seetharam, G., and Saville, B.A., "Degradation of Phenol Using Tyrosinase Immobilized on Siliceous Supports", *Wat. Res.*, 37, 436-440 (2003).
28. Saville, B.A., Khavkine, M., Seetharam, G., Marandi, B., and Zuo Y-L., "Characterization and Performance of Immobilized Amylase and Cellulase", *Applied Biochemistry and Biotechnology*, Vol 113-116, 251-259 (2004).
29. Zuo, Y-L. and Saville, B.A., "Efficacy of Immobilized Cellulase for Deinking of Mixed Office Waste", *Journal of Pulp and Paper Science*, 31(1), 3-6 (2005).
30. Saville, B.A., Huang, C, Yacyshyn, V., Desbarats, A., "Properties and Performance of Glucoamylases for Fuel Ethanol Production", *Applied Biochemistry and Biotechnology*, Vol 133(1) (2006).

31. Jeng, R., Huang, C., Sain, M., Hubbes, M., and Saville, B.A., "Production of starch-like exopolysaccharide using the filamentous fungus *Ophiostoma ulmi*", accepted in J. Industrial Crops and Products (2006)
32. Sain, M, Jeng, R., Saville, B., Huang, C., and Hubbes, M., "Starch-Based Polymer Conversion through Filamentous Fungi Fermentation", Biotechnology Focus, 16-18, April (2006).

#### **Refereed Manuscripts, submitted**

- 1) Saville, B.A., and Yacyshyn, V., "Effect of Cellulase Supplementation on Cookline Operation in Dry Mill Ethanol Plants", submitted to *Process Biochemistry*
- 2) Wang, G., Saville, B.A., Pelton, R., and Sodhi, R., "Use of Principal Component Analysis (PCA) to Improve ToF-SIMS Observation of Filler Distribution in Paper", submitted to *Analyst*.
- 3) Lim, C, Saville, B.A., "Thermostoinactivation Mechanism of Glucose Isomerase", accepted in *Applied Biochemistry and Biotechnology*.
- 4) Huang, C., Jeng, R., Saville, B.A., Sain, M., Hubbes, M., "Production, characterization and mechanical properties of starch modified by *Ophiostoma ulmi*, accepted in *Bioresources*.

#### **Refereed Books and Book Chapters**

- 1) R.W. Missen, C.A. Mims, and B.A. Saville: **An Introduction to Chemical Reaction Engineering and Kinetics**, 672pp, John Wiley and Sons (1999); includes E-Z Solve Software, Instructors' Resource Toolkit, and Solutions Manual on CD-ROMs.
- 2) S. Friedrich, B.A. Saville, and Y-L. Cheng, "Mathematical Modelling of Drug Distribution in the Vitreous Humour", invited book chapter in **Ophthalmic Drug Delivery Systems**, Volume II. Marcel Dekker (2003).
1. Borghei-Ghomi, R., Saville, B.A. "Xylanase for Pulp Biobleaching", National Silicates Ltd., 1999.
2. van Hellemond, E., Saville, B.A. "Techniques for Large Scale Recovery of Immobilized Enzymes", Advanced Biochemical Corporation, 2001.
3. Seetharam, G., Saville, B.A., "Comparison of  $\alpha$ -Amylase Performance: Influence of Immobilization Conditions on Activity", Advanced Biochemical Corporation, 2002.
4. Jono, V., Saville, B.A., Pelton, R.H. "High Efficiency Paper Additives Based on Enzyme-Recognition Functions", Surface Science Consortium, 2002.
5. Ali, N., Lim, C., Saville, B.A., Reeve, D.W. "Chemical Characterization of Surfaces of Pulp Fibers", Surface Science Consortium, 2002.
6. Saville, B.A., Mahinpey, N. "Mathematical Modelling of Statim Boiler Performance", SciCan, 2002.
7. Saville, B.A. "Mathematical Modelling of Steam Generation In the Statim Boiler", SciCan, 2002.
8. Saville, B.A. "Effect of Cellulase Supplementation on Cookline Performance, Advanced Biochemical Corporation and Alchem LLLP., 2002.
9. Saville, B.A. "Performance of a Novel Amylase for Starch Hydrolysis, Immortazyme and Alchem LLLP, 2003



10. Saville, B.A., Gibson, J. "Denco Advantase™ Trials", DENCO LLC and Immortazyme, 2004.
11. Poon, M.K., Saville, B.A. "Biophysical Characterization of  $\alpha$ -Amylases", Immortazyme, 2004.
12. Saville, B.A. "Technical Assessment of the Performance and Function of Commercial Glucoamylases", Alchem LLP and Immortazyme., 2004.
13. Saville, B.A. "Assessment of Enerzyme Performance Relative to Various Commercial Glucoamylases: Summary of Results from Glucoamylase Studies", Immortazyme, 2004.
14. Saville, B.A. "Characterization and Technical Assessment of Catalytically-Modified Alpha Amylases", Immortazyme, 2004.
15. Duff, B., Saville, B.A., Evaluation of the Feasibility and Potential Synergy of Integrating Corn Oil Extraction and Biodiesel Production at a Dry Mill Fuel Ethanol Plant", Natural Resources Canada, September, 2005
16. Saville, B., and Yancey, M., "Feasibility of a Biofuels Facility in Southeast Missouri", Bootheel AgriEnergy, October, 2005.
17. Desbarats, A., Saville, B.A., "Glucoamylase Trial Summary", Immortazyme and Western Plains Energy, 2005
18. Saville, B.A., "Feasibility of a Biofuels Facility in Durham", Regional Municipality of Durham, 2006
19. Saville, B.A., "Technical Analysis of Cookline and Fermentation Operations at Commercial Alcohols", Commercial Alcohols Inc., 2006.

#### **Conference Manuscripts**

1. Warrington J.C., and Saville, B.A. "Tyrosinase Performance and Inactivation in Organic Solvents", Conference Proceedings, AIChE 1999 Annual Meeting, Dallas. (NSERC)
2. Nazari H. and Saville: B.A. "Enhancement of Glucose Oxidase Stability by Co-immobilization with Catalase on Nylon 6,6", Conference Proceedings, AIChE 1999 Annual Meeting, Dallas. (NSERC)
3. Kutacova, P. Saville, B.A. Roy, D.N. Chatterjee, A.: "Enzymatic Modification of Kenaf Pulp", Conference Proceedings, AIChE 1999 Annual Meeting, Dallas. (NSERC)
4. Marandi, B., Saville, B.A., and Reeve, D.W. "Assessment of Wood Fibers using Labelled Enzymes", International Congress of Biotechnology in the Pulp and Paper Industries, Helsinki, 2001. (Surface Science Consortium)

#### **Presentations and Abstracts** (asterisk indicates invited lecture)

1. Jono, V., Saville, B.A., Pelton, R.H. "High Efficiency Paper Additives Based on Enzyme Recognition Functions", published in the proceedings of the Murray Moo-Young Biotechnology Symposium, Sept 5-7, 2002, Waterloo, Ontario. (Surface Science Consortium)
2. Zuo, Y-L., Saville B.A., "Efficacy of Immobilized Enzymes to Deink Mixed Office Wastes, published in the proceedings of the Murray Moo-Young Biotechnology Symposium, Sept 5-7, 2002, Waterloo, Ontario. (NSERC)
3. Saville, B.A., Khavkine, M., Seetharam, G., Marandi, B., Zuo, Y-L. "Characterization and Performance of Immobilized Amylase and Cellulase", 25<sup>th</sup> Symposium on

- Biotechnology for Fuels and Chemicals, Breckenridge, CO, May 4-7, 2003, p41. (Advanced Biochemical Corp.)
4. Lim, L-H., Huang, C. and Saville, B.A., "Stability and Performance of Glucose Isomerase for the Production of High Fructose Corn Syrup", CSCHE Conference, Hamilton, 2003 (Advanced Biochemical Corp.).
  5. Spensieri, C., Saville, B.A., "A Novel Enzymatic Approach to Soil and Groundwater Remediation", CSCHE Conference, Calgary, 2004 (NSERC)
  6. Wang, G., Saville, B.A., and Pelton, R.H., "Use of ToF-SIMS to Characterize Paper Surfaces and Fillers" CSCHE Conference, Calgary, 2004 (Surface Science Consortium, NSERC).
  7. Jeng, R., Huang, C., Sain, M., Hubbes, M., and Saville, B.A., "Production of Starch-Based Polymers Using the Filamentous Fungus *Ophiostoma Ulmi*", BIOCAP Canada National Conference, Ottawa, 2005 (BIOCAP, NSERC)
  8. Saville, B.A., Yacyshyn, V., "Effect of Cellulase Supplementation on Cookline Operation in Dry Mill Ethanol Plants", CSCHE Conference, Toronto, 2005 (Immortazyme)
  9. Lim, C., Saville, B.A., "Study of High Fructose Corn Syrup Production with Glucose Isomerase" CSCHE Conference, Toronto, 2005 (Immortazyme, NSERC).
  10. Wang, G., Poon, G., and Saville, B.A., "Oxalate Oxidase Characterization and Kinetics", 28<sup>th</sup> Symposium on Biotechnology for Fuels and Chemicals, Nashville, 2006. (Immortazyme).
  11. Lim, C., Saville, B.A., "Deactivation Mechanisms of Glucose Isomerase" 28<sup>th</sup> Symposium on Biotechnology for Fuels and Chemicals, Nashville, 2006. (Immortazyme, NSERC).
  12. V. Jono, B.A. Saville, R.H. Pelton, "Intelligent Paper Additives", CPPA Conference, Montreal, January 2002.
  13. V. Jono, B.A. Saville, R.H. Pelton, "Paper Additives Based on Enzyme Recognition Functions", Ontario-Quebec Biotechnology Conference, Ottawa, June 2002.
  14. Y-L. Zuo and B.A. Saville, "Immobilized Enzymes to Deink Mixed Office Waste", Ontario-Quebec Biotechnology Conference, Ottawa, June 2002.
  15. G. Wang, V. Jono, B.A. Saville, and R.H. Pelton, "High Efficiency Paper Additives Based on Enzyme Recognition Functions", Presentation to Domtar, August, 2003.
  16. M.K. Poon, B.A. Saville and V. Yacyshyn, "Purification and Analysis of Active  $\alpha$ -Amylase by Sephadex Chromatography", Ontario-Quebec Biotechnology Exchange, Laval, QC, June, 2004
  17. M.K. Poon, B.A. Saville and V. Yacyshyn, "Purification and Structural Characterization of  $\alpha$ -Amylases Wageningen-Toronto University Exchange, June, 2004.
  18. M. Yancey and B.A. Saville, "Feasibility of an Ethanol Production Facility in Southeast Missouri", Cape Girardeau, MO, October, 2005.
  19. M. Yancey, B. Duff, B.A. Saville, "Evaluating the Feasibility and Potential Synergy of Integrating Corn Oil Extraction and Biodiesel Production at a Dry Mill Ethanol Plant, Eastern Region Biofuels Workshop, Atlanta, GA, October, 2005.
  20. M. Bryan, B. Duff, B.A. Saville, "Evaluating the Feasibility and Potential Synergy of Integrating Corn Oil Extraction and Biodiesel Production at a Dry Mill Ethanol Plant, Canadian Renewable Fuels Conference, Toronto, ON, December, 2005.

Attachment #2: List of patents and patent applications

1. B.A. Saville, G.N. LeFevre: "Enzyme Immobilization on a Siliceous Support with a Polyaldehyde Cross-linking Agent", U.S. Patent 5,998,183, (1999).
2. B.A. Saville, G.N. LeFevre: "Enzyme Immobilization on a Siliceous Support with a Polyaldehyde Cross-linking Agent", Canadian Patent 2,277,371.
3. B.A. Saville, "Recovery Method for Immobilized Biocatalysts", Canadian Patent Application 2,354,782, August, 2001.
4. B.A. Saville, "Recovery Method for Immobilized Biocatalysts", U.S. Application 10/200,166; PCT Application PCT/CA02/01126, July 2002.
5. B.A. Saville, "Recovery Method for Immobilized Biocatalysts", South African Patent issued September, 2004.
6. B.A. Saville, "Recovery Method for Immobilized Biocatalysts", Patent Applications in China (02815225.5), Europe (0275014.4), and Australia (20022355759), January 2004
7. B.A. Saville, R.H. Pelton, "Paper Additives for Pulp and Paper Processing", Canadian Patent Application 2,415,911, January, 2003.
8. B.A. Saville and M. Khavkine, Enhancement of Enzyme Activity by Selective Purification, Canadian Patent 2,421,832
9. B.A. Saville and M. Khavkine, Enhancement of Enzyme Activity by Selective Purification, U.S. Application 10/797,019; PCT Application CA2004/00037, March, 2004
10. B.A. Saville and M. Khavkine, Enhancement of Enzyme Activity Through Purification and Immobilization, Canadian Patent Application 2,421,829, March, 2003.
11. B.A. Saville and M. Khavkine, Enhancement of Enzyme Activity Through Purification and Immobilization, U.S. Application 10/797,020 and PCT Application CA2004/000354, 2004.
12. B.A. Saville, R.H. Pelton, "Paper Additives for Pulp and Paper Processing", U.S. Application 10/871,031, June, 2004.